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# CONCERNING TANGENTS TO CONTINUA IN THE PLANE

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The line $k$ is said[1] to be tangent to the non-degenerate continuum[2] $M$ at the point $P$ of $M$ if every subset of $M$ which has $P$ as a limit point intersects every domain which contains $k-P$ and is the sum of the interiors of two vertical angles with vertex at $P$.

The straight line ray $PB$ is said to be tangent to the non-degenerate continuum $M$ at the point $P$ of $M$ if every subset of $M$ which has $P$ as a limit point intersects every domain which contains $B$ and is the interior of an angle with vertex at $P$.

THEOREM 1. *No continuum $M$ contains an uncountable point set $H$ such that for each point $P$ of $H$ there is a ray starting from $P$ and tangent to $M$ at $P$.*

*Proof.* Suppose there exists a continuum $M$ containing such a point set $H$. If $P$ is a point of $H$ there exist an acute angle $\alpha$ with vertex at $P$ and a domain $D$ containing $P$ such that $M \cdot D - P$ lies in the interior of $\alpha$. It follows, by a theorem of Denjoy's, that $H$ is countable.

THEOREM 2. *If the non-degenerate continuum $M$ has a tangent at each of its points then every subcontinuum of $M$ is a continuous curve.*

*Proof.* Suppose $N$ is a non-degenerate subcontinuum of $M$. Clearly every tangent to $M$ at a point of $N$ is also tangent to $N$ at that point. Let $K$ denote the set of all points $P$ of $N$ such that no ray starting from $P$ is tangent to $N$ at $P$. Every point of $K$ is a local separating point of $N$. Hence, by Theorem 1 and a theorem of G. T. Whyburn's,[3] $N$ is a continuous curve.

THEOREM 3. *If the compact continuum $M$ has a tangent at each of its points and $K$ is the set of all emanation points of triods lying in $M$ then $\bar{K}$ is totally disconnected.*

*Proof.* Suppose, on the contrary, that $\bar{K}$ contains a nondegenerate continuum and therefore an arc $t$. Since every point of $t$ is a limit point of $K$, every one is a limit point of a subset $H$ of $K$ such that every point of $H$

is the emanation point of some simple triod lying wholly in $t$ except for one arc that has only $P$ in common with $t$. Suppose $P_1$ is a point of $H$. There exist three arcs $P_1X_1$, $P_1X_2$ and $P_1X_3$ lying in $M$ such that two of them lie on $t$ and the third one has only $P_1$ in common with $t$ and no two of them have in common with each other any point except $P_1$. Let $P_1A$ and $P_1B$ denote the two rays which start from $P_1$ and lie on the straight line which is tangent to $M$ at $P_1$. There exists a rectangle $CDEF$ of diameter less than 1 such that (1) its diagonals $CE$ and $DF$ intersect at $P_1$, (2) every point of $M-P_1$ that lies in its interior lies in the interior of one of the two triangles $CP_1D$ and $EP_1F$, (3) $X_1$, $X_2$ and $X_3$ are in its exterior and (4) the angle $CP_1D$ of the triangle $CP_1D$ is acute. For each $n$ less than 4 let $Y_n$ denote the first point in the order from $P_1$ to $X_n$ that the arc $PX_n$ has in common with the perimeter of $CDEF$. Each of the points $Y_1$, $Y_2$ and $Y_3$ lies on one of the straight line intervals $CD$ and $EF$. Hence there exist points $C_1$, $D_1$, $W_1$ and $Z_1$ such that (1) $C_1$ and $D_1$ are either $C$ and $D$ or $E$ and $F$ and $W_1$ and $Z_1$ are two of the points $Y_1$, $Y_2$ and $Y_3$ and (2) $W_1$ and $Z_1$ both lie on $C_1D_1$, (3) $Z_1$ belongs to $t$. Let $P_1W_1$ denote the interval, with end-points at $P_1$ and $W_1$, of that one of the arcs $P_1X_1$, $P_1X_2$ and $P_1X_3$ on which $W_1$ lies and let $P_1Z_1$ denote the interval, with end-points at $P_1$ and $Z_1$, of the one on which $Z_1$ lies. The arcs $P_1W_1$ and $P_1Z_1$ lie, except for their end-points, wholly in the interior of the triangle $C_1P_1D_1$.

Similarly, in view of the fact that $P_1Z_1$ is a subset of $t$, it is clear that there exist a triangle $C_2P_2D_2$ of diameter less than $1/2$ and arcs $P_2Z_2$ and $P_2W_2$ lying in $M$ such that (1) if $P$ is any point in the interior of the triangle $C_2P_2D_2$ the distance from $P$ to $P_1$ is less than one half the distance from $P$ to the line $C_1D_1$, (2) the angle $C_2P_2D_2$ of this triangle is less than one half of the angle $C_1P_1D_1$ of the triangle $C_1P_1D_1$, (3) the triangle $C_2P_2D_2$ lies wholly in the interior of the triangle $C_1P_1D_1$, (4) $P_2$ belongs to $H$ and the arc $P_2Z_2$ is a subset of the arc $P_1Z_1$ and (5) $W_2$ and $Z_2$ lie on the interval $C_2D_2$ and each of the arcs $P_2Z_2$ and $P_2W_2$ lies, except for its end-points, wholly in the interior of the triangle $C_2P_2D_2$. This process may be continued. It follows that there exist an infinite sequence of triangles $C_1P_1D_1$, $C_2P_2D_2$, . . . and two infinite sequences of arcs $P_1W_1$, $P_2W_2$, . . . and $P_1Z_1$, $P_2Z_2$, . . . such that $C_1P_1D_1$, $P_1Z_1$ and $P_1W_1$ are as described above and, if for each $n$, the angle $C_nP_nD_n$ of the triangle $C_nP_nD_n$ is called $\alpha_n$ and the interior of this triangle is called $I_n$ then, for each $n$, (1) the diameter of $I_n$ is less than $1/n$ and $I_{n+1}$ is a subset of $I_n$, (2) the angle $\alpha_{n+1}$ is less than one-half of the angle $\alpha_n$, (3) the distance from each point of $I_{n+1}$ to the line $C_nD_n$ is more than $n$ times the distance from that point to $P_n$, (4) the arcs $P_nZ_n$ and $P_nW_n$ lie in $M$ and, except for their end-points, in $I_n$ and the points $Z_n$ and $W_n$ lie on the interval $C_nD_n$. The point sets $I_1$, $I_2$, $I_3$, . . . have a point $O$ in common. Suppose $h$ and $k$ are two straight lines through $O$ and suppose there exist infinitely many positive integers $n$ such that $h$

and $k$ both intersect the interval $C_nD_n$. Then there exist two rays $OL$ and $OT$ lying on $h$ and $k$, respectively, and such that, for infinitely many integers $n$, $OL$ and $OT$ both intersect $C_nD_n$ and therefore the acute angle $C_nOD_n$ is not less than the acute angle $LOT$. In view of conditions (2) and (3) this is clearly impossible. Hence there exists a line $\delta$ through $O$ such that no other line through $O$ intersects more than a finite number of the intervals of the sequence $C_1D_1$, $C_2D_2$, . . . . Suppose $x$ is a line through $O$ distinct from $q$. There exists a number $\delta_x$ such that, for every $n$ greater than $\delta_x$, $x$ contains no point of the side $C_nD_n$, and therefore intersects both of the sides $P_nC_n$ and $P_nD_n$ of the triangle $C_nP_nD_n$. It follows that, for each such $n$, $x$ intersects both of the arcs $P_nZ_n$ and $P_nW_n$ in the interior of $C_nP_nD_n$ and thus contains two points of $M$ (and therefore a point of $M$ distinct from $O$) in the interior of that triangle. Thus every line through $O$ except $q$ contains a subset of $M$ having $O$ as a limit point. But this is impossible since there is a tangent to $M$ at $O$.

THEOREM 4. *If the closure of the compact and countable point set $K$ is totally disconnected then there exists a dendron $M$ such that* (1) *$K$ is the set of all junction points of $M$ and* (2) *$M$ is topologically equivalent to a dendron that has a tangent at every one of its points.*

*Proof.* Since $\bar{K}$ is totally disconnected and $K$ is compact and countable there exists an arc $\alpha$ containing $\bar{K}$ and such that (1) every point of $K$ is a limit point of some component of $\alpha - \bar{K}$ and (2) if $K$ is nondegenerate the end-points of $\alpha$ belong to $\bar{K}$. There exists a reversibly continuous transformation $T$ of the plane into itself such that $T(\alpha)$ is a straight line interval $AB$. Let $P_1, P_2, \ldots$ denote the points of $T(K)$ and for each $n$ let $K_n$ denote one component of $AB - T(\bar{K})$ of which $P_n$ is a limit point. For each $n$, $K_n$ is a straight line segment having $P_n$ as one of its end-points. There exists a sequence of mutually exclusive circles $\beta_1, \beta_2, \beta_3, \ldots$ such that (1) for each $n$, $\beta_n$ is tangent, at $P_n$, to the straight line that joins $A$ and $B$, (2) the length of the segment $K_n$ is greater than $n$ times the diameter of $\beta_n$. For each $n$ let $\gamma_n$ denote an arc of $\beta_n$ such that (1) one of its end-points is at $P_n$, (2) its length is less than one-fourth of the length of $\beta_n$, (3) its orthogonal projection onto $AB$ is a subset of $P + K_n$. Let $N$ denote the point set $AB + \gamma_1 + \gamma_2 + \ldots$. This point set is a dendron with a tangent at each of its points and $K$ is the set of all junction points of the dendron of which $N$ is the image under the transformation $T$.

THEOREM 5. *If the compact continuum $M$ has a tangent at every one of its points and $K$ is the set of all points $P$ of $M$ such that there is a straight line ray starting from $P$ and tangent to $M$ there then $K$ is a countable inner limiting set and its closure is totally disconnected.*

*Proof.* Let $K_1$ denote the set of all end-points of $M$. The set $K_1$ is a subset of $K$. Let $K_2$ denote $K - K_1$. By Theorem 1, $K_2$ is countable. By a theorem of Menger's,[4] $K_1$ is an inner limiting set. Suppose $K_2$ is not.

Then it contains a point set $K_3$ which is dense in itself. Let $P_1$ denote some point of $K_3$. Let $P_1O_1$ denote the ray starting from $P_1$ which is tangent to $M$ at that point. Let $R_1$ denote a circular domain with center at $P_1$ and diameter 1. There exists an isosceles triangle $C_1P_1D_1$ lying, together with its interior $I_1$, in $R_1$ and such that the point $O_1$ is within the acute angle $C_1P_1D_1$ of this triangle and no point of $M$ except $P_1$ is on either of the straight line intervals $P_1C_1$ and $P_1D_1$ but $M$ contains two arcs $P_1Z_1$ and $P_1W_1$ having only $P_1$ in common and lying wholly in $I_1$ except for the point $P$ and the points $Z_1$ and $W_1$ both of which lie on the interval $C_1D_1$. There exists a domain $R_2$ lying in $R_1$ and containing $P_1$ but no point of $M$ that is not in the interior of the angle $C_1P_1D_1$. Since $P_1$ is a limit point of $K_3$, this domain contains a point $P_2$ of $K_3$ distinct from $P_1$ and at a distance from $P_1$ less than half its distance from the line $C_1D_1$. The point $P_2$ belongs to $I_1$. If $P_2O_2$ is the straight line ray which is tangent to $M$ at $P_2$, and which starts at that point, there exists an isosceles triangle $C_2P_2D_2$, lying with its interior $I_2$ wholly in $I_1$ and within a circle with center at $P_2$ and diameter $1/2$, such that (1) $O_2$ lies within the angle $C_2P_2D_2$ of this triangle and no point of $M$ except $P_2$ is on either of the straight line intervals $P_2C_2$ and $P_2D_2$ but $M$ contains two arcs $P_2Z_2$ and $P_2W_2$ having only $P_2$ in common and lying wholly in $I_2$ except for $P_2$ and the points $Z_2$ and $W_2$ which lie on the interval $C_2D_2$, (2) the angle $C_2P_2D_2$ is less than one half the angle $C_1P_1D_1$ and (3) the distance from any point of $I_2$ to the line $C_1D_1$ is more than twice its distance from $P_1$. This process may be continued. It follows that there exist an infinite sequence of triangles $C_1P_1D_1$, $C_2P_2D_2$, . . . and two infinite sequences of arcs $P_1Z_1$, $P_2Z_2$, . . . and $P_1W_1$, $P_2W_2$, . . . such that $C_1P_1D_1$ and $P_1Z_1$ and $P_2Z_2$ are as indicated above and such that if, for each $n$, $I_n$ denotes the interior of the triangle $C_nP_nD_n$ and $\alpha_n$ denotes the angle $C_nP_nD_n$ of this triangle then, for each $n$, there hold true four conditions which may be worded precisely as are Conditions (1)–(4) concerning the $I_n$, $\alpha_n$, $P_nZ_n$, $P_nW_n$, $C_nP_nD_n$, etc., with which the above proof of Theorem 3 is concerned. A contradiction follows as in that proof. Hence $K_2$ is an inner limiting set. Since both $K_1$ and $K_2$ are inner limiting sets so is $K$, their sum.

Suppose now that $K$ contains a nondegenerate continuum. Then it contains an arc $t$. Since no subset of $K$ is dense in itself, therefore there exists an interval $t_1$ of $t$ which contains no point of $K$. The interval $t_1$ is a continuum of condensation of $M$ and therefore, if $H$ is the set of all emanation points of simple triods lying in $M$, $t_1$ is a subset of the closure of $H$ (and indeed of the closure of $H \cdot t_1$). But this is contrary to Theorem 3.

[1] Cf. Besicovitch, A. S., *Fund. Math.*, **22**, 49–56 (1934).

[2] Throughout this paper space is supposed to be the plane.

[3] Whyburn, G. T., "Sets of Local Separating Points of a Continuum," *Bull. Amer. Math Soc.*, **39**, 97–100 (1933).

[4] Menger, Karl, "Grundzüge einer Theorie der Kurven", *Math. Annalen*, **95**, 272–306 (1925).

# TORUS HOMOTOPY GROUPS*

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A promising line of attack on the unsolved problem of calculating the homotopy groups $\pi_r$, $r = 1, 2, \ldots$, of a topological space $Y$ was initiated by J. H. C. Whitehead.[1] The results which he has obtained involve a certain "multiplication": for any pair of elements $\alpha \in \pi_m$, $\beta \in \pi_n$ the Whitehead product $\alpha \cdot \beta$ is defined and is an element of the group $\pi_{m+n-1}$. This multiplication can be compared with the group operation only when $m = n = 1$ and in this case $\alpha \cdot \beta = \alpha\beta\alpha^{-1}\beta^{-1}$.

In order to study this product I define here, for every dimension $r \geqslant 1$, a group $\tau_r$ which I call the $r$-dimensional torus homotopy group and which has the following properties:

1. Every homotopy group of dimension less than $r + 1$ can be mapped isomorphically into $\tau_r$. (In fact most homotopy groups have many such isomorphisms.)

2. If $\gamma = \alpha \cdot \beta$, where $\alpha \in \pi_m$, $\beta \in \pi_n$, $\gamma \in \pi_{m+n-1}$ and $m + n - 1 < r + 1$, then isomorphisms $\pi_m \to \tau_r$, $\pi_n \to \tau_r$, $\pi_{m+n-1} \to \tau_r$ can be so chosen that $\alpha \to \bar{\alpha}$, $\beta \to \bar{\beta}$, $\gamma \to \bar{\gamma}$ and $\bar{\gamma} = \bar{\alpha}\bar{\beta}\bar{\alpha}^{-1}\bar{\beta}^{-1}$.

The groups $\tau_1, \tau_2, \tau_3, \ldots$ form a direct homomorphism system and the limit group $\tau$ has properties (1) and (2) with $r = \infty$. Thus all the homotopy groups and all the Whitehead products can be studied within one single group $\tau$.

Let $Y$ be a topological space and $y_*$ one of its points. Denote by $T_{r-1}$ the $(r-1)$-dimensional torus whose coordinates are the $r - 1$ real numbers $x_1, x_2, \ldots, x_{r-1}$ (mod 1). The $r$-dimensional torus homotopy group $\tau_r(Y) = \tau_r(Y, y_*)$ is defined to be the fundamental group of the function space $Y^{T_{r-1}}$, using the mapping $y_* = y_*^{T_{r-1}}$ as base point. Thus an element of $\tau_r(Y)$ is represented by a continuous $Y$-valued function $f$ of $r$ real variables $x_0, x_1, \ldots, x_{r-1}$ (mod 1) which satisfies the condition

$$(*) \qquad f(x_0, x_1, \ldots, x_{r-1}) = y_* \text{ if } x_0 \equiv 0.$$

Denoting by $\mathfrak{L}_r$ the totality of such functions $f$, it is easy to see that two such functions $f_0$ and $f_1$ determine the same element of $\tau_r$ whenever there is a homotopy $f_t$, $0 \leq t \leq 1$, between them such that $f_t \in \mathfrak{L}_r$ for every $t$. The multiplication in $\tau_r$ is determined by the multiplication in $\mathfrak{L}_r$: $h = f \cdot g$ is defined by

$$h(x_0, x_1, \ldots, x_{r-1}) = \begin{cases} f(2x_0, x_1, \ldots, x_{r-1}) \text{ when } 0 \leq x_0 \leq 1/2, \\ g(2x_0 - 1, x_1, \ldots, x_{r-1}) \text{ when } 1/2 \leq x_0 \leq 1. \end{cases}$$

The discussion of the preceding paragraph parallels a discussion of the classical homotopy groups[2] which is presumedly known to the reader. It is sufficient here to recall that an element of $\pi_m(Y) = \pi_m(Y, y_*)$ is represented by a continuous $Y$-valued function $f$ of $m$ real variables $x_0, x_1, \ldots, x_{m-1}$ (mod 1) which satisfies the condition

(**) $\quad f(x_0, x_1, \ldots, x_{m-1}) = y_*$ if $x_i \equiv 0$ for some $i = 0, 1, \ldots, m-1$.

Let the totality of such functions $f$ be denoted by $\mathfrak{F}_m$. If $m \leq r$ and $A$ denotes a sequence $\{i_1, i_2, \ldots, i_{m-1}\}$, where $0 < i_1 < \ldots < i_{m-1} < r$, then, by assigning to every $f \in \mathfrak{F}_m$ that $g \in \mathfrak{L}_r$ which is defined by

$$g(x_0, x_1, \ldots, x_{r-1}) = f(x_0, x_{i_1}, x_{i_2}, \ldots, x_{i_{m-1}}),$$

a homomorphism $\omega_r^A$: $\alpha \to \alpha^A$ of $\pi_m(Y)$ into $\tau_r(Y)$ is set up. These homomorphisms can be shown to be isomorphisms, although the proof of this fact is by no means easy. There are exactly $\binom{r-1}{m-1}$ of these isomorphisms $\pi_m \to \tau_r$, and there is no reason for preferring any of them over the others. It is easily seen that $\tau_1$ is identical with $\pi_1$, and that $\tau_2$ is identical with Abe's[3] group $\kappa_2$.

A homomorphism $\Phi$ of $\tau_r$ onto $\tau_{r-1}$ is defined by assigning to every $f \in \mathfrak{L}_r$ that $g \in \mathfrak{L}_{r-1}$ which is defined by

$$g(x_0, x_1, \ldots, x_{r-2}) = f(x_0, x_1, \ldots, x_{r-2}, 0).$$

It can be shown that the subgroup $\tau_r'$ of $\tau_r$ which is generated by the subgroups $\omega_r^A(\pi_m)$, $2 \leq m \leq r'$, $A \subset \{1, 2, \ldots, r-1\}$, is the direct product $\mathbf{X}\omega_r^A(\pi_m)$ of these subgroups, that $\tau_r'$ is the nucleus of the homomorphism $\Phi$ and that $\tau_r$ is a split extension of the abelian subgroup $\tau_r'$ by $\tau_{r-1}$.

In general the torus homotopy groups are non-abelian. In particular $\tau_r$ is non-abelian in the case that $Y$ is the union of an $m$-sphere and an $n$-sphere ($m + n - 1 = r$) which have exactly one point in common. Thus $\tau_r$ may be non-abelian for simply connected spaces if $r \geq 3$ and for spaces with abelian fundamental group if $r \geq 2$. This shows that not all of the "non-abelian character" of a space is expressed by its fundamental group. However if $Y$ is a topological group its torus homotopy groups are all abelian.

The exact statement of how the Whitehead products are represented in $\tau_r$ as commutators is as follows: *If $A$ has $m-1$ indices and $B$ has $n-1$ indices, if $A \frown B$ is vacuous and if $\alpha \in \pi_m(Y)$ and $\beta \in \pi_n(Y)$ then* $(\alpha \cdot \beta)^{A \sim B} = [\alpha^A, \beta^B]^{\epsilon\eta}$, *where $[u, v]$ denotes the commutator $uvu^{-1}v^{-1}$; $\epsilon = (-1)^{n-1}$ and $\eta = (-1)^w$ where $w$ is the number of instances of $i > j$ with $i \in A$ and $j \in B$.* It should be observed that it follows from previous discussion that $[\alpha^A, \beta^B] = 1$ if $A \frown B$ is not vacuous. By the theory of group-extensions

it can be shown that $\tau_r$ is determined by the groups $\pi_1, \pi_2, \ldots, \pi_r$ and the Whitehead products $\alpha \cdot \beta$, $\alpha \in \pi_m$, $\beta \in \pi_n$, $m + n - 1 < r + 1$.

By restating in terms of torus homotopy groups the combination of two of J. H. C. Whitehead's results[1] we find the following theorem: *Let $K$ be a complex obtained from a complex $K^*$ by removing the interior $\sigma - \dot{\sigma}$ of a principal $n$-dimensional simplex $\sigma$, where $n > 2$. The nucleus of the injection homomorphism $\tau_n(K) \to \tau_n(K^*)$ is precisely the invariant subgroup of $\tau_n(K)$ which is generated by the image of the injection homomorphism $\tau_n(\dot{\sigma}) \to \tau_n(K)$.* This reformulation seems to have more intuitive content and suggests more clearly than the original theorems an attack on the harder problem where $\dim \sigma > n$.

If $Y_*$ is a subset of $Y$ which contains $y_*$ the relative torus homotopy group $\tau_r(Y \bmod Y_*, y_*)$, $r \geq 2$, may be defined. One starts with the collection $\mathfrak{L}_r(Y \bmod Y_*, y_*)$, of those continuous $Y$-valued functions $f$ of the $r - 1$ real variables $x_0, x_1, \ldots, x_{r-2} \pmod 1$ and the real variable $0 \leq t \leq 1$ which satisfy the conditions

$$(***) \qquad \begin{cases} f(x_0, x_1, \ldots, x_{r-2}, t) \in Y_* \text{ if } t = 0, \\ f(x_0, x_1, \ldots, x_{r-2}, t) = y_* \text{ if } t = 1 \text{ or if } x_0 \equiv 0. \end{cases}$$

From $\mathfrak{L}_r(Y \bmod Y_*, y_*)$ the group $\tau_r(Y \bmod Y_*, y_*)$ is obtained by the same procedure which led to the definition of $\tau_r(Y, y_*) = \tau_r(Y \bmod y_*, y_*)$. Just as in the absolute case there are isomorphisms $\omega_r^A$: $\pi_m(Y \bmod Y_*) \to \tau_r(Y \bmod Y_*)$, where $A \subset \{1, 2, \ldots, r-2\}$, and homomorphisms $\Phi$: $\tau_r(Y \bmod Y_*) \to \tau_{r-1}(Y \bmod Y_*)$. The subgroup $\tau_r'(Y \bmod Y_*)$ of $\tau_r(Y \bmod Y_*)$ which is generated by the subgroups $\omega_r^A(\pi_m(Y \bmod Y_*))$, $m \geq 3$, $A \subset \{1, 2, \ldots, r-2\}$, is the direct product of these subgroups, $\tau'$ is the nucleus of $\Phi$ and $\tau$ is a split extension of $\tau_r'$ by $\tau_{r-1}$

A homomorphism of $\tau_{r+1}(Y \bmod Y_*)$ into $\tau_r(Y_*)$ is defined by the transformation $f \to f \mid \{t = 0\}$; a homomorphism of $\tau_r(Y_*)$ into $\tau_r(Y)$ is defined by injection of $Y_*$ into $Y$; a homomorphism of $\tau_{r+1}'(Y)$ into $\tau_{r+1}(Y \bmod Y_*)$ is defined by $f \to g$ where $g(x_0; x_1, \ldots, x_{r-1}; x_r) = f(x_0, x_A)$, $A \subset \{1, 2, \ldots, r\}$, $2 \leq m \leq r + 1$. In the resulting system of homomorphisms

$$\tau_{r+1}'(Y) \to \tau_{r+1}(Y \bmod Y_*) \to \tau_r(Y_*) \to \tau_r(Y)$$

it may be verified that the nucleus of any homomorphism is the image of the preceding homomorphism and that the nucleus of the first homomorphism is generated by the images of the injection homomorphisms $\pi_m(Y_*) \to \pi_m(Y)$, $2 \leq m \leq r + 1$. If $Y$ is a fibre space over a space $Z$ and $Y_*$ is the fibre which contains $y_*$ and whose image point is the base point $z_*$ of $Z$ then $\tau_r(Y \bmod Y_*, y_*) \approx \tau_r(Z, z_*)$ and the homomorphism system becomes

$$\tau_{r+1}'(Y) \to \tau_{r+1}(Z) \to \tau_r(Y_*) \to \tau_r(Y).$$

Any element of $\tau_r$ determines, together with the fundamental $r$-cycle of the antecedent space, a continuous $r$-dimensional cycle. In this fashion a homomorphism $\tau_r(Y)$ into the $r$-dimensional homology group $H_r(Y)$ of $Y$ is defined. The image group is the group of spherical $r$-dimensional cycles, and the nucleus of this homomorphism obviously contains the commutator subgroup of $\tau_r$. However, it is not true that the commutator subgroup is the nucleus. This is shown by the example of the 3-sphere $Y = S^3$ with $r = 4$. It is known that there is a non-trivial element $\alpha$ of $\pi_4(S^3)$, hence $\alpha^A$ is an element of the nucleus; on the other hand $\tau_4(S^3)$ is abelian because $S^3$ is a group manifold.

Elements of the nucleus which are not commutators may be constructed with the help of the homotopy groups of the rotation groups. If $\alpha \in \pi_n(Y)$ and $\rho$ is an element of $\pi_m(R_{n-1})$, where $R_{n-1}$ is the rotation group of the $(n-1)$-sphere then there is determined an element $\alpha^\rho \in \tau_{m+n}(Y)$. The element $(\alpha^\rho)^A(\alpha^{-1})^B$, where $A = \{1, 2, \ldots, m+n-1\}$ and $B = \{1, 2, \ldots, n-1\}$, belongs to the nucleus of the homomorphism $\tau_{m+n}(Y) \to H_{m+n}(Y)$. If $\alpha$ is the generator of $\pi_2(S^2)$ and $\rho$ is the generator of $\pi_1(R_1)$ then $(\alpha^\rho)^A(\alpha^{-1})^B$ is the generator of $\pi_3(S^2)$. From the fact that the "Einhängung" of this element is non-trivial it can be shown that $(\alpha^\rho)^A(\alpha^{-1})^B$ is not a commutator.[4]

The torus homotopy groups have a rather obvious generalization which may turn out to be useful. Let $\mu$ be any mapping of $T_{r-1}$ into $Y$ and consider the fundamental group $\tau_r(Y, \mu)$ of the function space $Y^{T_{r-1}}$ using the mapping $\mu$ as base point. If $\mu = y_*$ the definition reduces to that of $\tau_r(Y, y_*)$. The group $\tau_r(Y, \mu)$ depends only on $r$, $Y$ and the homotopy class of $\mu$; particularly, to any element $\alpha$ of $\tau_r(Y, \mu)$ there is a group $\tau_{r+1}(Y, \alpha)$. The cycles which arise from the homomorphism of $\tau_r(Y, \mu)$ into $H_r(Y)$ need not be spherical cycles; for example, the fundamental cycle of the torus can occur.

A more detailed analysis of the torus homotopy groups will appear elsewhere.

* Presented to the American Mathematical Society under the title "The Complete Homotopy Group," abstracts 49-11-306(1943) and 51-1-49 (1945).

[1] *Ann. Math.*, **42**, 409–428 (1941) and *Proc. London Math. Soc.*, **45**, 243–327 (1939), §§10, 11.

[2] All homotopy groups are multiplicative in this paper.

[3] *Jap. Jour. Math.*, **16**, 169 (1940).

[4] In this and the succeeding paragraph there is a certain amount of overlapping with recent unpublished work by George W. Whitehead.

# EXPERIMENTS ON SEXUAL ISOLATION IN DROSOPHILA. IV. MODIFICATION OF THE DEGREE OF ISOLATION BETWEEN DROSOPHILA PSEUDOOBSCURA AND DROSOPHILA PERSIMILIS AND OF SEXUAL PREFERENCES IN DROSOPHILA PROSALTANS

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Sexually active animals find their potential mates and recognize them as belonging to the same species with the aid of stimuli that are but poorly known. In birds, it has been shown that species recognition may be either strictly innate or conditioned through experience (for a short summary of the literature see Cushing[1]). Conditioning seems to play a major rôle particularly in species with highly developed parental care. On the other hand, innate mechanisms control the recognition of potential mates of the same species in birds that are not raised by their own parents, such as cowbirds, parasitic cuckoos and megapodes. The same seems to be true for most of the lower vertebrates and invertebrates. It is, however, very little known to what extent the functioning of the innate patterns may be influenced by conditioning and by other extrinsic factors. The experiments described below were devised to explore the possibilities in this field.

*Material and Methods.*—For most of the experiments an orange-eyed mutant strain of *Drosophila pseudoobscura* Frolova descended from flies collected at Piñon Flats, San Jacinto Mountains, California, and a wild strain of *Drosophila persimilis* Dobzhansky and Epling from Stony Creek, north of the Sequoia National Park, California, were used. For some of the experiments strains of *Drosophila prosaltans* Duda from Chilpancingo and Zopilote Canyon, Mexico, and from Belem, Iporanga, and Bertioga, Brazil, were employed (concerning these strains see Dobzhansky and Streisinger[2]).

The two species, *D. pseudoobscura* and *D. persimilis*, are almost indistinguishable morphologically, although Reed, Williams and Chadwick[3] were able to discriminate the strains at their disposal with the aid of an ingeniously contrived ratio of thorax volume to the product of wing area times the cubed wing length. *D. persimilis* was formerly known as "*D. pseudoobscura* race B." The irrationality of this designation became progressively more clear with the accumulation of data showing that these species are distinct genetic systems; their separation is fully maintained in nature despite the broad overlapping of their distribution areas.[4] Too great an emphasis on morphological distinctions as species criteria leads to results that are plainly untenable. One would have to break living mankind into five species that are not at all isolated reproductively,[5] and yet consider as

single "species" some groups of clearly separate species of Drosophila.[6]

Except when stated otherwise, the experimental procedure was the same as described in the preceding parts of this series.[2, 7] Batches of ten freshly hatched females of each of two species or strains to be tested were placed with ten males of one of these species in vials with food. After 4 to 7 days, depending on species and the particular experiment, the females were dissected and their sperm receptacles and spermathecae examined for sperm. The amounts of sperm present in an inseminated female vary greatly, particularly in heterogamic crosses: sometimes the ventral receptacle is tightly filled with sperm, sometimes loosely, and sometimes only a few moving spermatozoa are found. No attempt was made to record the various degrees of insemination; so long as any sperm was found, the fly was recorded as inseminated. In only a single *D. pseudoobscura* female, sperm was found in the spermatheca but none in the receptacle. The stock bottles were kept mostly at room temperature, and the experimental vials in an incubator at 24½°C. Since the experiments lasted from May to November, "room temperature" varied considerably, and this may be a source of error in some of the experiments.

Sexual isolation between *D. pseudoobscura* and *D. persimilis* was first discovered by Lancefield[8] and subsequently studied by Boche,[9] but the data of the last-named investigator have never been published. We found the isolation to be much stronger when *D. pseudoobscura* than when *D. persimilis* males are used; it must, however, be noted that our experiments concern a single strain of each species, and that other strains may quite conceivably behave differently. Such differences between strains of *D. pseudoobscura* and *D. persimilis* with respect to sexual isolation from a third species, namely, *D. miranda*, are, indeed, known.[10]

*Mixed Cultures.*—Specific smells may be very important components of isolating mechanisms in animals with a highly developed olfactory sense. Experiments were, therefore, arranged to test whether or not there was a difference in the degree of sexual isolation between *D. pseudoobscura* and *D. persimilis* when these flies are raised in separate culture bottles or together in the same bottle. Fertilized females of the two species were placed together, but without males, in the same culture bottle, and transferred to fresh bottles at about 24-hour intervals. The larvae of both species grew up together in the same culture medium. The flies of the two species were separated after hatching before any copulation could occur. Sets of 10 females of both species were then confined with 10 males of one or the other species in vials with food, whereupon the females were dissected and their seminal receptacles were examined for sperm. As a control, similar tests were made using flies of the two species which developed in separate bottles.

It can be seen from table 1 that raising flies of the two species in the same culture medium does not lower the sexual isolation between them; curiously

enough, the results seem to indicate, if anything, the opposite. It probably occurs in nature not infrequently that larvae of the sympatric species *D. pseudoobscura* and *D. persimilis* grow up in the same food medium, and it is obviously of survival value to both species that the mixing of the smells of their larvae does not lead to lowering of isolation between the two species.

TABLE 1

NUMBER OF FEMALES DISSECTED (*n*) AND PER CENT CARRYING SPERM (%) IN CROSSES IN WHICH *D. pseudoobscura* AND *D. persimilis* FLIES WERE RAISED TOGETHER IN THE SAME BOTTLE OR IN DIFFERENT BOTTLES

| MALE | RAISED | HOMOGAMIC *n* | HOMOGAMIC % | HETEROGAMIC *n* | HETEROGAMIC % | $\chi^2$ | ISOLATION INDEX |
|---|---|---|---|---|---|---|---|
| pseudoobscura | separately | 82 | 87.8 | 83 | 9.6 | 100.7 | 0.80 |
| pseudoobscura | together | 145 | 89.6 | 140 | 0.7 | 226.6 | 0.98 |
| persimilis | separately | 127 | 63.8 | 119 | 22.7 | 42.5 | 0.48 |
| persimilis | together (May–June) | 41 | 73.2 | 41 | 2.4 | 43.6 | 0.94 |
| persimilis | together (July) | 82 | 76.5 | 89 | 32.9 | 31.2 | 0.40 |

*Conditioning.*—A set of *D. pseudoobscura* males was divided in two parts; some males were kept for 8–15 days in regular culture bottles with an excess of females of their own species ("pro-conditioned"), and others for the same length of time with females of *D. persimilis* ("counter-conditioned"). Similarly, some *D. persimilis* males were "pro-conditioned" and others were "counter-conditioned." Groups of 10 males were, then, confined with 10 freshly hatched females of each of the two species in vials with food; the females were dissected and examined for sperm. In "control" experiments freshly hatched males were confined with freshly hatched females. The results are summarized in table 2.

TABLE 2

NUMBER OF FEMALES DISSECTED (*n*) AND PER CENT CARRYING SPERM (%) IN CROSSES OF *D. pseudoobscura* AND *D. persimilis*

| MALES | HOMOGAMIC *n* | HOMOGAMIC % | HETEROGAMIC *n* | HETEROGAMIC % | $\chi^2$ | ISOLATION INDEX |
|---|---|---|---|---|---|---|
| pseudoobscura control | 82 | 87.8 | 83 | 9.6 | 100.7 | 0.80 |
| pseudoobscura pro-conditioned | 97 | 81.4 | 93 | 0.0 | 129.0 | 1.00 |
| pseudoobscura counter-conditioned | 115 | 88.7 | 122 | 1.6 | 182.7 | 0.96 |
| persimilis control | 127 | 63.8 | 119 | 22.7 | 42.5 | 0.48 |
| persimilis pro-conditioned | 32 | 56.3 | 37 | 13.5 | 13.9 | 0.69 |
| persimilis counter-conditioned | 47 | 87.2 | 52 | 38.5 | 25.0 | 0.39 |

The results are inconclusive as far as *D. pseudoobscura* males are concerned. There seems to be less isolation among the controls than among the counter-conditioned flies. However, the "control" experiment employed freshly hatched males while in the conditioning experiments males were 8–15 days old. Furthermore, most of the control experiments were made in May while the conditioning experiments were made in July. In

the case of *D. persimilis* males the conditioning appears to be effective. Males that had been conditioned with their own females show a higher isolation index (0.69) than the controls (0.48) or males conditioned with *D. pseudoobscura* females (0.39). The $\chi^2$ of the difference between control and pro-conditioned flies is 2.08 (for two degrees of freedom $P > 0.20$); the $\chi^2$ of the difference between control and counter-conditioned flies is 13.63 ($P < 0.01$). The effects of counter-conditioning are more significant than those of pro-conditioning.

*Light.*—Philip, Rendel, Spurway and Haldane[11] have stated that *D. subobscura*, a European relative of *D. pseudoobscura* and *D. persimilis*, does not mate in the absence of light, and that normal females of this species kick off mutant males with a yellow body color. Although morphological differences between *D. pseudoobscura* and *D. persimilis*, as well as those between strains of *D. prosaltans*, are very slight, the possibility that visual stimuli are involved in mate recognition is not excluded. To test this, vials were prepared containing two kinds of females and a single kind of males.

TABLE 3

MATE DISCRIMINATION IN THE LIGHT AND IN THE DARK

| LIGHT OR DARK | FEMALES | MALES | HOMOGAMIC n | HOMOGAMIC % | HETEROGAMIC n | HETEROGAMIC % | ISOLATION INDEX |
|---|---|---|---|---|---|---|---|
| Light | pseudoobscura, persimilis | pseudoobscura | 40 | 80.0 | 40 | 7.5 | 0.83 |
| Dark | pseudoobscura, persimilis | pseudoobscura | 60 | 80.0 | 69 | 2.9 | 0.93 |
| Light | pseudoobscura, persimilis | persimilis | 100 | 78.0 | 100 | 40.0 | 0.32 |
| Dark | pseudoobscura, persimilis | persimilis | 100 | 93.0 | 100 | 60.0 | 0.22 |
| Light | prosaltans-A, prosaltans-D | prosaltans-A | 70 | 82.9 | 65 | 3.1 | 0.93 |
| Dark | prosaltans-A, prosaltans-D | prosaltans-A | 69 | 46.4 | 68 | 1.5 | 0.94 |
| Light | prosaltans-B, prosaltans-C | prosaltans-B | 68 | 39.7 | 68 | 2.9 | 0.86 |
| Dark | prosaltans-B, prosaltans-C | prosaltans-B | 59 | 18.6 | 57 | 0.0 | 1.00 |

Some of the vials prepared on each of the days during which the experiments lasted were placed in an opaque box and the others on the top of the same box; the box was exposed to daylight but protected from direct sunlight. The temperature varied in the environment, but it was obviously very similar inside and outside the box. Females of the "dark series" were dissected soon after being removed from the box. The results are summarized in table 3; in this table the strains of *D. prosaltans* coming from Chilpancingo, Zopilote, Belem and Bertioga are denoted "prosaltans-A," B, C and D, respectively.

It is evident that in *D. pseudoobscura*, *D. persimilis* and *D. prosaltans* the mate discrimination is not greatly influenced by the presence or absence of light. In *D. prosaltans* the light has, however, an obvious influence on the total number of inseminations taking place within a given time interval; a significantly greater number of matings takes place in the vials exposed to light than in those kept in the dark. The data for *D. persimilis*,

if taken at their face value, would indicate an opposite effect of light, but the differences observed are in need of confirmation.

*The Role of the Wings.*—When a courting male of *D. pseudoobscura* or *D. persimilis* pursues a female he spreads and vibrates his wings. The pitch of vibration may be correlated with the wing surface[9] which is larger in the latter than in the former species. If females exercise the choice, it is possible that they are helped in recognition of the males by the pitch of the wing vibration. If so, the females should have difficulties in recognizing wingless males, and the isolation index should drop. Actually, the opposite happened when wingless *D. persimilis* males were confined with winged *D. persimilis* and *D. pseudoobscura* females—the isolation index became higher (table 4). However, the point when around 50 per cent of the females were inseminated was not reached after 4–5 days' exposure, as with normal males, but only after 8 days. Females seem to recognize the species of wingless males as readily as of normal ones, but either succeed better in avoiding insemination by not conspecific wingless males or are less easily excited into a receptive state.

TABLE 4

INSEMINATION RECORDS OF *D. persimilis* AND *D. pseudoobscura* FEMALES BY WINGLESS *D. persimilis* MALES

| HOMOGAMIC | | HETEROGAMIC | | | ISOLATION |
|---|---|---|---|---|---|
| *n* | % | *n* | % | $\chi^2$ | INDEX |
| 78 | 65.4 | 70 | 12.8 | 42.8 | 0.67 |

Experiments with wingless females and normal males resulted in isolation indices which are practically identical with the control experiments. This is important if considered in conjunction with the observation that non-receptive females often flick off with their wings males which attempt to mount them. Non-receptive wingless females seem to be equally capable of avoiding males.

*Sexual excitement.*—Dobzhansky and Koller,[10] working with *D. pseudoobscura* and *D. miranda*, obtained an indication that males aged in the absence of females are less efficient in discriminating between their own and foreign females than males pro-conditioned with their own females. If significant, this result may be due either to sexual excitement of the males aged without females or to the pro-conditioning of the other group of males. We have kept males and females of *D. pseudoobscura*, *D. persimilis*, and of four strains of *D. prosaltans* in isolation from individuals of the opposite sex but with abundant food for approximately seven days, whereupon these fully mature flies were placed together in the same vial, always avoiding etherization of the flies. As in the earlier experiments, one kind of males and two kinds of females were placed in each vial. The difference between this technique and that of Dobzhansky and Koller[10] lies in that in the experi-

ments under consideration both males and females were aged in isolation, while in the latter experiments only the males were so aged. Courting and copulating pairs can be seen in the vials within a few minutes after the flies are placed together, and in from one to four hours approximately half of all females are found to be inseminated. If freshly hatched flies are used, it takes from four to five days for half of the females to become inseminated, and relatively few copulating pairs are seen in the vials at any one time. The results of the experiments are summarized in table 5. The

TABLE 5

MATE DISCRIMINATION IN INDIVIDUALS AGED IN ISOLATION FROM THE OPPOSITE SEX

| FEMALES | MALES | HOMOGAMIC n | HOMOGAMIC % | HETEROGAMIC n | HETEROGAMIC % | $\chi^2$ | ISOLATION INDEX |
|---|---|---|---|---|---|---|---|
| pseudoobscura, persimilis | pseudoobscura | 40 | 70.0 | 37 | 5.4 | 20.53 | 0.85 |
| pseudoobscura, persimilis | persimilis | 43 | 67.4 | 56 | 7.1 | 26.65 | 0.81 |
| prosaltans-A, prosaltans-D | prosaltans-A | 63 | 93.6 | 74 | 23.0 | 30.50 | 0.61 |
| prosaltans-B, prosaltans-E | prosaltans-E | 38 | 2.6 | 43 | 67.4 | 22.63 | −0.92 |

Chilpancingo, Zopilote, Bertioga and Iporanga strains of *D. prosaltans* are referred to in this table as "prosaltans-A," B, D and E, respectively. Frequencies of the homogamic and heterogamic fertilizations in mixtures of *D. pseudoobscura* and *D. persimilis* shown in table 2 may be taken as control values for comparison with the data in table 5, although these experiments have not been performed simultaneously. For insemination records in *D. prosaltans* see Dobzhansky and Streisinger.[2]

Examination of table 5 shows that aging in the absence of individuals of the opposite sex fails to change the degree of sexual isolation when *D. pseudoobscura* males are used; with *D. persimilis* males, such aging leads even to a strengthening of the isolation, although more data are needed to establish this point. Aged prosaltans-A (Chilpancingo) males gave a somewhat lower isolation index than was obtained with males placed together with their prospective mates shortly after their hatching from the pupae; males of prosaltans-E (Iporanga strain) prefer B (Zopilote) females to their own, and this preference seems to be enhanced by aging in the absence of mates.

*Temperature.*—Flies raised at room temperature were placed in vials soon after their hatching from pupae, and the vials with the flies were kept at 24½°, 21°, 18° and 16½°C. for as long as necessary to obtain insemination of about half of the females. This takes 4–5 days at the higher and 7–9 days at the lower temperatures. The results are summarized in table 6. "Prosaltans-A," B, C and D are, in this table, the Chilpancingo, Zopilote, Belem and Bertioga strains of *D. prosaltans*, respectively.

The behavior of *D. pseudoobscura* and *D. prosaltans* flies is about the same at all the temperatures tried. *D. persimilis* shows clear sexual isola-

tion from *D. pseudoobscura* at the higher temperatures, but at 18° and 16½° *D. persimilis* males seem to discriminate against females of their own species in favor of those of *D. pseudoobscura*. This is particularly astonishing because *D. persimilis* is, on the whole, confined in nature to cooler habitats than *D. pseudoobscura*. It may be that females of *D. persimilis* become sexually receptive only very slowly at temperatures of 18°C. and lower, so that most *D. persimilis* females in the low temperature experiments were simply unavailable for insemination. To test this possibility, *D. persimilis* females and males, and *D. pseudoobscura* females, were aged for 10 days at 16½°C., and placed together, at the same temperature, for about 24

TABLE 6

MATE DISCRIMINATION AT DIFFERENT TEMPERATURES

| | | | HOMOGAMIC | | HETEROGAMIC | | | ISOLATION |
|---|---|---|---|---|---|---|---|---|
| t° | FEMALES | MALES | n | % | n | % | $\chi^2$ | INDEX |
| 24½° | pseudoobscura, persimilis | pseudoobscura | 30 | 83.3 | 28 | 3.6 | 20.4 | 0.92 |
| 18° | pseudoobscura, persimilis | pseudoobscura | 21 | 85.7 | 18 | 0.0 | 15.4 | 1.00 |
| 16½° | pseudoobscura, persimilis | pseudoobscura | 42 | 92.9 | 40 | 12.5 | 24.2 | 0.76 |
| 24½° | pseudoobscura, persimilis | persimilis | 65 | 93.8 | 64 | 39.1 | 14.6 | 0.41 |
| 21° | pseudoobscura, persimilis | persimilis | 56 | 53.6 | 63 | 12.7 | 15.4 | 0.62 |
| 18° | pseudoobscura, persimilis | persimilis | 21 | 4.8 | 20 | 55.0 | 8.7 | −0.84 |
| 16½° | pseudoobscura, persimilis | persimilis | 86 | 32.6 | 90 | 52.2 | 4.0 | −0.23 |
| 24½° | prosaltans-A, prosaltans-C | prosaltans-A | 59 | 86.4 | 58 | 8.6 | 37.8 | 0.82 |
| 24½° | prosaltans-B, prosaltans-D | prosaltans-B | 58 | 74.1 | 58 | 8.6 | 30.1 | 0.79 |
| 16½° | prosaltans-A, prosaltans-C | prosaltans-A | 77 | 90.9 | 75 | 2.3 | 62.3 | 0.94 |
| 16½° | prosaltans-B, prosaltans-C | prosaltans-B | 84 | 44.0 | 85 | 4.7 | 14.6 | 0.81 |

hours. Dissection of the females showed that 59.4% of the 106 *D. persimilis*, and 44.6% of the 92 *pseudoobscura* females were inseminated. This gives a non-significant positive isolation index of 0.14. Sexual isolation between *D. pseudoobscura* and *D. persimilis* appears, then, to be weaker at lower than at higher temperatures if males of *D. persimilis* are used.

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[1] Cushing, John E., Jr., *The Condor*, **43**, 233–236 (1941).
[2] Dobzhansky, Th., and Streisinger, G., these PROCEEDINGS, **30**, 340–345 (1944).
[3] Reed, S. C., Williams, C. M., and Chadwick, L. E., *Genetics*, **27**, 349–361 (1942).
[4] Dobzhansky, Th., and Epling, C., *Carnegie Inst. Washington, Publ.* **554**, 1–46 (1944).
[5] Gates, R. R., *Amer. Jour. Phys. Anthrop.*, **2** (n.s.), 279–292 (1944).
[6] Sturtevant, A. H., *Univ. Texas Publ.*, **4213**, 5–51 (1942).
[7] Dobzhansky, Th., and Mayr, E., these PROCEEDINGS, **30**, 238–244 (1944).
[8] Lancefield, D. E., *Zeits. ind. Abst. Vererbungsl.*, **52**, 287–317 (1929).
[9] Boche, R. D., in Dobzhansky's *Genetics and the Origin of Species*, New York, 1937.

[10] Dobzhansky, Th., and Koller, P.Ch., *Biol. Zentralb.*, **58**, 589–607 (1938).

[11] Philip, U., Rendel, J. M., Spurway, H., and Haldane, J. B. S., *Nature*, **154**, 260–262 (1944).

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## *DOMINANCE MODIFICATION AND PHYSIOLOGICAL EFFECTS OF GENES**

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Several years ago[1] there was found in the house mouse a mutation with several striking effects including absence or shortening of the tail, absence or abnormality of one or both kidneys, absence of external anus and genital aperture and abnormalities of other parts of the urogenital system. In the stock in which it occurred this whole syndrome of effects behaved as a unit and showed simple segregation from normal. The mutation acted as a lethal, all homozygotes *Sd Sd* being tailless and dying shortly after birth, all showing imperforate anus and absence of both kidneys. Heterozygotes showed a lesser expression of these defects, having short tails, and less severe urogenital malformations. The mutation in the original stock therefore acted as a dominant in respect to its effect on the tail, as recessive or nearly so in its lethal effect, and as incompletely dominant in its effect on urogenital development.

When the *Sd* mutation was removed from the stock in which it originally occurred and was transferred by a series of successive backcrosses to another inbred normal stock, the tail length of heterozygotes progressively decreased until after five backcross generations nearly all were tailless, while the viability of the heterozygotes decreased, due to the greater effect of *Sd* on the urogenital system.[1] About 90 per cent of all *Sd*+ animals at birth had abnormal kidneys.[2] The dominance of *Sd* on tail length appeared to have been increased by the genetic constitution of the new stock while the lethal effect appeared also to have become partially dominant. There was no evidence of necessary connection between the effect upon tail length and upon viability.

Since the above observations were published we have transferred the *Sd* mutation to two other normal-tailed inbred stocks by repeated backcrossing. In one of these stocks (identified as *m*) the tail length of heterozygotes increased, and the proportion of tailless animals among the heterozygotes decreased. In the $F_1$, $BC_1$ and $BC_2$ generations the cross of *Sd*+ by normal *m* produced 142 normal, 90 short-tailed and 25 tailless; while in $BC_3$ and $BC_4$ the comparable figures are 39 normal, 23 short and no tailless. The

totals, 181 normal and 138 $Sd+X^{2-5.26}_{p=0.2}$, indicate a *lowered* viability of *Sd+* associated with *increasing* tail length. This was probably due to increased severity of the urogenital lesions, since of four heterozygotes dissected at birth, three had urogenital abnormalities.

When backcrossed to the other inbred stock, a normal albino stock known as CF (Carworth Farms), changes in the opposite direction occurred in the *Sd* heterozygotes; that is, the tails became shorter, the proportion of tailless heterozygotes *increased*, and the relative viability of the heterozygotes *increased*. The figures are $F_1$–$BC_2$: 59 normal, 14 short, 53 tailless; $BC_3$–$BC_5$: 26 normal, 7 short, 18 tailless. The totals of 85 ++ 92 *Sd+* indicate no excess mortality of short and tailless *Sd+*. Forty-three of these heterozygotes were dissected and in all cases the urogenital system was normal. Thus the CF genetic constitution, which *increased* the severity of the tail defect, eliminated the deleterious effect of *Sd+* on viability and on the urogenital defects chiefly responsible for the viability effects of *Sd*. The conclusion is obvious that the several effects of *Sd* upon the heterozygotes are modified by *different* genetic factors.

Since this is so, the conclusions of Fisher and Holt[4] concerning dominance modification of *Sd* will have to be examined critically. Fisher and Holt similarly outcrossed *Sd+* animals obtained from this laboratory to other stocks in the Cambridge University laboratory, and set up selection lines in one of which the tail length of heterozygotes was increased, while in the other, tail length remained short. The viability of heterozygotes in the longer-tailed line improved. Fisher and Holt supposed that the chief factor in viability was tail length, so that natural selection for viability aided the selection for longer tails, while in the negative line it was "always acting in opposition, in that mice most defective in the development of the caudal vertebrae will also, on the whole, be most defective in other respects." This assumption, as our observations show, is entirely unwarranted. They assume also that as the tail length of the heterozygotes increases "so one might expect the chance of homozygotes becoming less abnormal, with a consequent lengthening of life, to increase." This is based on the same reasoning as that above and is supported only by the occurrence in the line selected for tail length, of two animals diagnosed as homozygotes (*Sd Sd*), one of which lived for 22 days and the other for 72 hours. Since tailless heterozygotes with internal lesions as severe as those of the longer lived assumed homozygote have been described by Gluecksohn-Schoenheimer,[2] the diagnosis of homozygosity rests on the occurrence of imperforate anus and cloaca. These defects have been found recently in rare cases among heterozygotes also, so it is possible that Fisher and Holt were dealing with an extreme heterozygote.

There is no doubt that the tail length of heterozygous *Sd+* mice is readily modified by other genetic factors, which thus may be said to modify the

dominance of *Sd* in its effect upon the tail. But these other factors, as our results show, may change the dominance of *Sd* in its other effects in ways opposite to that in which the tail expression is affected.

In order to derive some meaning from such observations for Fisher's general theory of the origin of dominance in evolution, it would be necessary to specify whether modifiers of *Sd* would be selected because of their effect on tail length or because of their effect on urogenital development. In view of the evident relation between the urogenital expression and viability there can be little doubt that modifiers which tend to make the urogenital effect of *Sd* recessive would be selected. But these same genetic constitutions may, as we have seen, increase the dominance of *Sd* in respect to tail length. It can be concluded that the evolutionary significance of factors affecting dominance can be properly assessed only when their physiological effects are known.

* The observations reported were made with the aid of a grant from the Josiah Macy, Jr., Foundation.

[1] Dunn, L. C., Gluecksohn-Schoenheimer, S., and Bryson, V., "A New Mutation in the Mouse Affecting Spinal Column and Urogenital System," *Jour. Heredity*, **31**, 343–348 (1940).

[2] Gluecksohn-Schoenheimer, S., "The Morphological Manifestations of a Dominant Mutation in Mice Affecting Tail and Urogenital System," *Genetics*, **28**, 341–348 (1943).

[3] Dunn, L. C., "Changes in the Degree of Dominance of Factors Affecting Tail-Length in the House Mouse," *The American Naturalist*, **76**, 552–569 (1942).

[4] Fisher, R. A., and Holt, S. B., "The Experimental Modification of Dominance in Danforth's Short-Tailed Mutant Mice," *Ann. Eugenics*, **12**, 102–120 (1944).

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## *RELATIVE SENSITIVITY OF CHROMOSOMES TO NEUTRONS AND X-RAYS. III. COMPARISON OF CARCINOMA AND LYMPHOSARCOMA IN THE RAT*

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In previous studies it was shown that the relative response of chromosomes of the "resting" nucleus to neutrons and x-rays ($n/x$) may be used as means for identifying physiological stages of the nucleus which cannot otherwise be recognized.[1, 2] By applying such an analysis to different tumors in the same species it is possible to determine whether the nuclei and chromosomes of the cell types so analyzed are in fact the same, as might be expected from existing theories in genetics and embryology, or

whether they differ. We wish to report here the results of an analysis of the Walker 256 carcinoma and a lymphosarcoma of the rat.

TABLE 1

WALKER CARCINOMA 256

| | X-RAYS | | | | NEUTRONS | | | |
|---|---|---|---|---|---|---|---|---|
| TIME IN HOURS | DOSE IN r UNITS | TOTAL ANA-PHASES | % NORMAL | COR-RECTED % NORMAL | DOSE IN n UNITS | TOTAL ANA-PHASES | % NORMAL | COR-RECTED % NORMAL |
| 3 | 47 | 569 | 61.5 | 66.5 | 8 | 708 | 62.7 | 67.7 |
| | 99 | 526 | 37.1 | 42.1 | 8.9 | 711 | 60.2 | 65.2 |
| | 143 | 568 | 30.1 | 35.1 | 12.5 | 584 | 48.9 | 53.9 |
| | 180 | 533 | 13.7 | 18.7 | 17.8 | 552 | 30.4 | 35.4 |
| | 240 | 539 | 7.9 | 12.9 | 27 | 613 | 17.3 | 22.3 |
| | 286 | 537 | 2.4 | 7.4 | 38 | 563 | 7.7 | 12.7 |
| | ... | ... | ... | ... | 41 | 740 | 7.4 | 12.4 |
| 8 | 50 | 606 | 69.1 | 74.1 | 9 | 518 | 61.7 | 66.7 |
| | 107 | 532 | 48.9 | 53.9 | 18 | 552 | 40.2 | 45.2 |
| | 164 | 681 | 36.3 | 41.3 | 24 | 616 | 30.2 | 35.2 |
| | 228 | 734 | 26.3 | 31.3 | 30 | 579 | 24.2 | 29.2 |
| | 277 | 692 | 17.1 | 22.1 | 44 | 580 | 15.7 | 20.7 |
| | 344 | 647 | 8.5 | 13.5 | 56 | 834 | 9.2 | 14.2 |
| 12 | 48 | 714 | 66.1 | 71.1 | 7 | 715 | 58.8 | 63.8 |
| | 98 | 608 | 47.6 | 52.6 | 21 | 545 | 31.6 | 36.6 |
| | 149 | 637 | 38.5 | 43.5 | 22 | 645 | 27.0 | 32.0 |
| | 192 | 846 | 28.9 | 33.9 | 37 | 547 | 15.2 | 20.2 |
| | 300 | 859 | 15.9 | 20.9 | 40 | 733 | 9.4 | 14.4 |
| | 337 | 797 | 11.4 | 16.4 | 54 | 516 | 4.8 | 9.8 |
| | 354 | 666 | 7.4 | 12.4 | ... | ... | ... | ... |
| | 450 | 655 | 4.6 | 9.6 | ... | ... | ... | ... |
| 18 | 120 | 671 | 63.6 | 68.6 | 6 | 565 | 75.9 | 80.9 |
| | 254 | 928 | 34.0 | 39.0 | 20 | 664 | 46.8 | 51.8 |
| | 392 | 536 | 24.8 | 29.8 | 36 | 575 | 26.1 | 31.1 |
| | 462 | 557 | 20.5 | 25.5 | 48 | 686 | 13.1 | 18.1 |
| | 588 | 527 | 10.1 | 15.1 | 49 | 597 | 11.9 | 16.9 |
| | 684 | 623 | 4.9 | 9.9 | 62.9 | 722 | 7.9 | 12.9 |
| | ... | ... | ... | ... | 63.7 | 572 | 6.6 | 11.6 |
| | ... | ... | ... | ... | 74.4 | 633 | 4.6 | 9.6 |
| | ... | ... | ... | ... | 81.7 | 675 | 1.7 | 6.7 |
| 24 | 95 | 583 | 69.6 | 74.6 | 18 | 627 | 55.8 | 60.8 |
| | 120 | 606 | 77.2 | 82.2 | 24 | 648 | 42.3 | 47.3 |
| | 239 | 627 | 44.6 | 49.6 | 43 | 584 | 26.9 | 31.9 |
| | 392 | 863 | 30.0 | 35.0 | 55 | 607 | 18.8 | 23.8 |
| | 462 | 577 | 27.9 | 32.9 | 92 | 658 | 5.9 | 10.9 |
| | 588 | 505 | 20.5 | 25.5 | 104 | 744 | 3.1 | 8.1 |
| | 784 | 637 | 10.8 | 15.8 | ... | ... | ... | ... |

The animals carrying the tumors were treated with x-rays or with neutrons by the same methods and apparatus previously described.[2] The methods for fixing the tissue, preparing the smears and making the counts have also been presented.

The data obtained from counts of normal and abnormal anaphases are presented in tables 1 and 2. For plotting the curves (Figs. 1, 2, 3 and 4) each per cent normal anaphases was corrected by adding to it the average per cent abnormal anaphases of all controls for that tumor. The correction for the carcinoma was 5.0%, the average from 12 control animals; for the lymphosarcoma it was 12.1%, the average from 7 controls. The per cent

TABLE 2

LYMPHOSARCOMA

| | X-RAYS | | | | NEUTRONS | | | |
|---|---|---|---|---|---|---|---|---|
| TIME IN HOURS | DOSE IN $\gamma$ UNITS | TOTAL ANA-PHASES | % NORMAL | CORRECTED % NORMAL | DOSE IN $n$ UNITS | TOTAL ANA-PHASES | % NORMAL | CORRECTED % NORMAL |
| 3 | 51 | 543 | 63.9 | 76.0 | 8 | 519 | 62.6 | 74.7 |
| | 97 | 538 | 43.1 | 55.2 | 16 | 518 | 47.8 | 59.9 |
| | 148 | 578 | 31.3 | 43.4 | 22 | 506 | 32.4 | 44.5 |
| | 201 | 513 | 17.6 | 29.7 | 34 | 504 | 18.6 | 30.7 |
| | 272 | 529 | 6.4 | 18.5 | 51 | 503 | 8.9 | 21.0 |
| | 341 | 543 | 2.8 | 14.9 | 58 | 521 | 2.6 | 14.7 |
| 8 | 96 | 545 | 56.9 | 69.0 | 10 | 601 | 68.5 | 80.6 |
| | 166 | 544 | 45.4 | 57.5 | 20.5 | 520 | 50.9 | 63.0 |
| | 226 | 526 | 28.5 | 40.6 | 31 | 540 | 37.2 | 49.3 |
| | 301 | 525 | 21.5 | 33.6 | 41 | 511 | 27.8 | 39.9 |
| | 365 | 524 | 15.1 | 27.2 | 51 | 516 | 18.6 | 30.7 |
| | 424 | 516 | 9.5 | 21.6 | 63 | 521 | 10.1 | 22.2 |
| 12 | 55 | 553 | 66.7 | 78.8 | 8 | 559 | 73.9 | 86.0 |
| | 117 | 555 | 52.0 | 64.1 | 21 | 517 | 45.6 | 57.7 |
| | 197 | 520 | 33.8 | 45.9 | 39 | 518 | 28.2 | 40.3 |
| | 274 | 524 | 25.2 | 37.3 | 45 | 524 | 23.6 | 35.7 |
| | 361 | 536 | 17.9 | 30.0 | 57 | 539 | 14.1 | 26.2 |
| | 417 | 543 | 12.3 | 24.4 | 67 | 522 | 12.4 | 24.5 |
| 18 | 95 | 556 | 55.8 | 67.9 | ... | ... | ... | ... |
| | 189 | 540 | 44.8 | 56.9 | ... | ... | ... | ... |
| | 295 | 634 | 24.9 | 37.0 | ... | ... | ... | ... |
| | 384 | 553 | 14.3 | 26.4 | ... | ... | ... | ... |
| | 484 | 539 | 8.7 | 20.8 | ... | ... | ... | ... |
| | 566 | 524 | 5.5 | 17.6 | ... | ... | ... | ... |
| 24 | 94 | 572 | 64.4 | 76.5 | 10 | 629 | 72.9 | 85.0 |
| | 206 | 596 | 44.9 | 57.0 | 22.5 | 550 | 58.2 | 70.3 |
| | 307 | 531 | 34.8 | 46.9 | 42 | 530 | 44.5 | 56.6 |
| | 414 | 526 | 27.3 | 39.4 | 51 | 546 | 37.0 | 49.1 |
| | 539 | 590 | 15.4 | 27.5 | 84 | 516 | 18.0 | 30.1 |
| | 637 | 545 | 12.1 | 24.2 | 89 | 540 | 13.5 | 25.6 |

abnormal anaphases in unirradiated animals is considerably greater in the lymphosarcoma than in any other type of tumor studied so far. When plotted semi-logarithmically the per cent normal anaphases for each time interval following irradiation fall on a straight line, i.e., the data fit an exponential curve. The points were all fitted graphically. The mean deviation of the points so fitted varied from 0.7 to 3.7%, with only

one curve giving a mean deviation greater than 3%, so the fit may be considered good. Table 3 gives the slopes of the curves, the mean deviation of the points about each curve, and the ratio $(n/x)$ of the slopes obtained with neutrons to those obtained with x-rays.

The slopes of the curves for the lymphosarcoma are consistently smaller than the corresponding slopes for the carcinoma for both x-rays and neu-

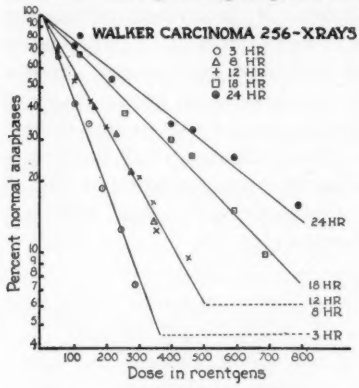


FIGURE 1

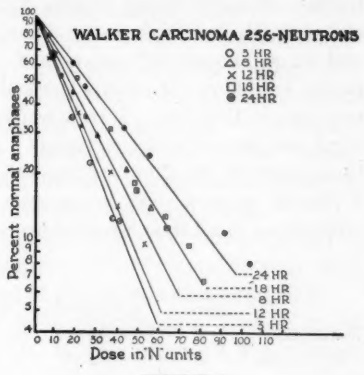


FIGURE 2

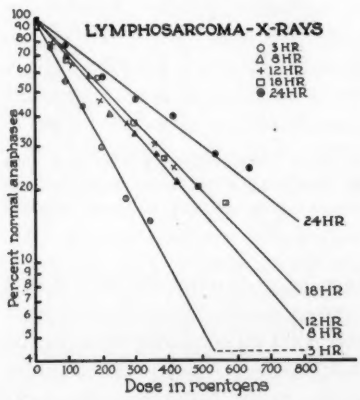


FIGURE 3

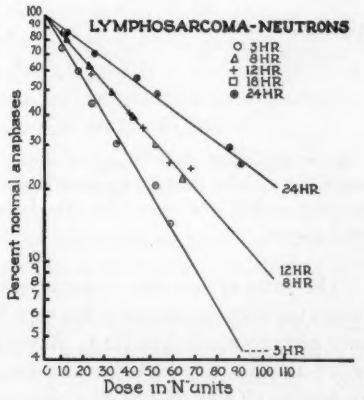


FIGURE 4

trons at all time intervals except at 24 hours. Such a result might be expected if the chromosomes of the lymphosarcoma were smaller than those of the carcinoma. However, the chromosomes of the cells studied showed no very obvious differences in size, although it was found impossible to make any accurate measurements. We cannot, therefore, offer any explanation for the observed differences in slopes.

Although there is a progressive decrease in the slopes observed following x-rays or neutrons in both tumors at 8, 18 and 24 hours after irradiation, the slopes at 12 hours are anomalous in this respect. In the lymphosarcoma there is no significant difference between the slopes at 8 and 12 hours, which is also true for the carcinoma treated with x-rays. The latter tumor when treated with neutrons, however, gives a steeper slope at 12 than at 8 or 18 hours. It is interesting to note that analysis of chromosome response in the plant *Vicia faba* shows no difference in x-ray slopes at 12 and 18 hours and no significant difference in 8- and 12-hour neutron slopes. In the mouse lymphoma there is a marked rise in the slope for both x-rays and neutrons at 12 hours. If slopes of survival curves for either x-rays or neutrons are plotted as a function of time after irradiation, in all the tissues thus analyzed the slope for the 12-hour interval appears either as part of a plateau or as a peak in the curve. Obviously the 12-hour stage is a critical one in all the chromosomes analyzed so far.

TABLE 3

| | TIME IN HOURS | $k_x \times 10^{-3}$ | $d_x$ | $k_n \times 10^{-3}$ | $d_n$ | $n/x$ |
|---|---|---|---|---|---|---|
| Walker 256 carcinoma | 3 | 8.45 | 1.8 | 50.81 | 2.0 | 6.01 |
| | 8 | 5.52 | 1.5 | 40.76 | 2.7 | 7.28 |
| | 12 | 5.58 | 2.3 | 49.27 | 2.9 | 8.83 |
| | 18 | 3.25 | 2.0 | 33.62 | 1.1 | 10.34 |
| | 24 | 2.49 | 3.7 | 26.93 | 2.0 | 10.81 |
| Lympho-sarcoma | 3 | 5.86 | 1.5 | 33.80 | 1.8 | 5.77 |
| | 8 | 3.65 | 1.5 | 22.73 | 0.7 | 6.23 |
| | 12 | 3.66 | 2.2 | 22.91 | 1.9 | 6.26 |
| | 18 | 3.32 | 1.3 | ... | ... | ... |
| | 24 | 2.44 | 2.2 | 14.62 | 1.6 | 5.99 |

$k_x$ = slopes following treatment with x-rays, $k_n$ = slopes following treatment with neutrons, $d_x$ = deviation of points from x-ray curves, $d_n$ = deviation of points from neutron curves, $n/x$ = ratio of the slopes obtained with neutrons to those obtained with x-rays.

The ratio of neutron to x-ray efficiency ($n/x$) is approximately 6 at 3 hours for both tumors, as it has been for chromosomes of all species of plants and animals studied so far.[3] Beyond this point all similarity between the $n/x$ ratios for the two tumors ceases. While there is a progressive increase in $n/x$ to 10.8 at 24 hours in the carcinoma, in the lymphosarcoma it remains at approximately 6 for all the periods studied.† However, the ratios for the lymphosarcoma are strikingly similar to those observed in a mouse lymphoma, for which the ratios were 5.8, 5.9, 8.8, 5.9, and 5.9 for the intervals 3, 8, 12, 18 and 24 hours.[2] In other words, the chromosomes of the rat lymphosarcoma in their reaction to x-rays and neutrons resemble those of the mouse lymphoma much more than they do chromosomes of another tumor of the rat.‡ For reasons previously given,[1, 2] the differences in re-

sponse of the chromosomes cannot be ascribed to differences in the extrachromosomal (cytoplasmic) conditions of the histologically different types of cells involved. Likewise the theory advanced by other investigators[4, 5] which postulates chromosome "breakage" followed by "healing" of the broken ends, cannot account for results such as these.[1] We must conclude, therefore, that there are physiological differences in the chromosomes of two different tissues (lymphoid and epithelial) of the same species.

Theories concerning cell differentiation during the early stages of ontogeny and of "de-differentiation" in carcinogenesis commonly imply that the genic constitution of the cells remains constant. By implication it is also inferred that the chromosomes also remain unaltered. The experiments reported here indicate that such assumptions are entirely unwarranted. Possible changes in the chromosomes during embryogeny or carcinogenesis may be detected by the methods described here.

*Summary.*—1. Chromosomes of the Walker 256 rat carcinoma are more sensitive to x-rays and neutrons than those of a rat lymphosarcoma in all parts of the resting stage studied except at 24 hours prior to anaphase.

2. In both these tumors, as well as in a previously studied mouse lymphoma and the plant *Vicia faba*, the mitotic phase 12 hours before anaphase appears to be a critical one.

3. The ratio of neutron to x-ray efficiency, $n/x$, which was previously shown to be an index of physiological activity of the chromosomes, is approximately 6 at all time intervals studied in the lymphosarcoma, whereas in the carcinoma $n/x$ increases progressively from 6.0 at 3 hours to 10.8 at 24 hours. From this it is concluded that there are physiological differences in the chromosomes of two different tissues in the same species.

4. Chromosome response of the rat lymphosarcoma to x-rays and neutrons is more like that of the mouse lymphoma than that of the rat carcinoma.

5. Results obtained here indicate that assumptions that the chromosomes remain unaltered during ontogeny of normal cells and during de-differentiation in carcinogenesis are unwarranted.

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† A breakdown in the cyclotron made it impossible to obtain the data needed for the 18-hour neutron curve. However, the argument presented here will not be altered by whatever value of $n/x$ may be obtained for this interval.

‡ Although the mouse lymphoma and rat lymphosarcoma do show similarity in response as determined by the ratio $n/x$, there is a difference between the two in another respect. When abnormalities are plotted as a function of time after irradiation, the mouse lymphoma chromosomes show a peak in sensitivity at 12 hours as well as at 3 hours, which does not appear in the lymphosarcoma.

[1] Marshak, A., *Proc. Nat. Acad. Sci.*, **28**, 29–35 (1942).

[2] Marshak, A., *Radiology*, **39**, 621–626 (1942).

[3] Marshak, A., *Proc. Soc. Exp. Biol. Med.*, **41**, 176–180 (1939).

[4] Gustafsson, A., *Hereditas*, **23**, 281–335 (1937).

[5] Sax, K., *Genetics*, **26**, 418–425 (1941).